REMARKS

Claims 2-34 remain pending in the instant application. Claims 18-34 are withdrawn from consideration as drawn to a non-elected invention. Accordingly, claims 2-17 remain under consideration in the instant application.

Support for the claim amendments can be found throughout the claims and specification as originally filed. No new matter has been added. Applicants reserve the right to prosecute the claims as originally filed in this or a continuing application.

Claims 18-34 (Group II) are related to claims 1-17 (Group I) as product and process of use. It is Applicant's understanding that, once the pending product claims are found allowable, any non-elected process claims (Group II, claims 18-34) will be rejoined and examined if such process claims include all of the limitations of the elected product claims (MPEP §821.04).

Acknowledgement of the Withdrawal of Previous Rejections

Applicants gratefully acknowledge the withdrawal of: (a) the previous rejection of claims 1-2, 4-5 and 10-17 under 35 U.S.C. 102(e) as being anticipated by Tuschl *et al.*; (b) the previous rejection of claim 3 under 35 U.S.C. § 102(e) as being anticipated by Xu *et al.*; and (c) the previous rejection of claims 3-5, 7 and 9 under 35 U.S.C. § 103(a) as being unpatentable over Xu *et al.* in view of Buhr *et al.*.

Rejection of Claims 3-17 under 35 U.S.C. § 112, First Paragraph

The Examiner has maintained the rejection of claims 3-17 as failing to comply with the written description requirement. In particular, the Examiner maintains that "despite the examples of disease caused by dominant, gain-of-function mutations and the general guidelines provided by [the] application, one of skill in the art cannot readily extrapolate these general teachings provided in the specification to adequately describe the entire genus of siRNA targeting any mutant gene that would *direct cleavage* of mRNA encoded by the mutant gene because the art teaches variability within the genus. The Examiner cites Holen *et al.* (Nucleic Acids Res. 2002) for teaching that "siRNAs directed against the same target varied widely in their silencing efficiencies."

Applicants respectfully traverse this rejection and reiterate below the arguments set forth in the previous Response filed February 15, 2007.

The fundamental factual inquiry in a written description rejection is whether the claim defines an invention that is clearly conveyed to those skilled in the art at the time the application was filed. The subject matter of the claim ***need not be described literally*** (i.e. using the same terms or in haec verba) in order for the disclosure to satisfy the written description requirement. MPEP 2163.02. Rather, the inquiry into whether the written description requirement is met must be determined on a case-by-case basis and is a question of fact. *In re Wertheim*, 541 F.2d 257, 262, 191 USPQ 90, 96 (CCPA 1976).

Moreover, the Federal Circuit in *Capon* has firmly established that the descriptive text needed to meet the Written Description requirement varies with the nature and scope of the invention at issue, and with the scientific and technologic knowledge already in existence. *Capon v. Eshhar*, 418 F.3d 1349, 1357 (Fed. Cir. 2005). In *Capon*, the Federal Circuit explained that "since the law is applied to each invention in view of the state of the relevant knowledge, ***its application will vary with differences in the state of knowledge in the field and differences in the predictability of the science***." *Id.* Specifically, the Court stated that:

> Precedent illustrates that the determination of what is needed to support generic claims to biological subject matter ***depends on a variety of factors, such as the existing knowledge in the particular field, the extent and content of the prior art, the maturity of the science or technology, the predictability of the aspect at issue, and other considerations appropriate to the subject matter***. *Id.* at 1359 (emphasis added).

The Court further explained that "the written description may be satisfied 'if in the knowledge of the art the disclosed function is sufficiently correlated to a particular, known structure.'" *Id.* (citing *Amgen Inc. v. Hoechst Marion Roussel*, Inc., 314 F.3d 1313, 1332 (Fed. Cir. 2003) (emphasis added)). Accordingly, "[a]s each field evolves, the balance also evolves between what is known and what is added by each inventive contribution." *Id.* at 1358.

Applicants submit that based on the foregoing considerations and framework for written description, particularly as articulated by the Federal Circuit in *Capon*, the subject matter of the pending claims is fully described in accordance with 35 U.S.C. §112, first paragraph, by the present specification.

Claim 3, and claims 4-17 which depend therefrom, are drawn to a small interfering RNA (siRNA) comprising a sense strand and an antisense strand, wherein the ***sense strand comprises a sequence homologous to a region of a mutant allele encoding a gain-of-function mutant protein***, said ***region comprising one or more point mutations***, and wherein the ***antisense strand comprises a sequence comprising one or more modified bases positioned opposite the point mutations***, such that the siRNA directs allele-specific cleavage of a mRNA encoded by the mutant allele.

The Examiner alleges that "the art does not provide a core structure or motif that would function in directing allele-specific cleavage of any mutant allele" and concludes that "one is left to empirically screen for siRNA compounds of the invention." Applicants respectfully submit that ***Applicants' specification,*** and not the art, ***provides the core structure*** of the presently claimed siRNAs that functions in directing allele-specific cleavage of any mutant allele. In particular, Applicants' specification teaches that the ***critical structural feature*** of the siRNAs of the invention is the ***presence in the antisense strand of a modified base positioned opposite a point mutation in a target mRNA*** encoded by a mutant allele. Given this ***core structural feature***, one of ordinary skill in the art is put in possession of a broad variety of siRNA compounds that direct allele-specific cleavage of an mRNA encoded by a mutant allele. The remaining structure, *i.e.*, sequence, of the siRNAs of the invention ***depends necessarily on the sequence of the particular target mRNA*** and, accordingly, ***cannot be shared*** by all the siRNAs of the invention.

The Examiner further alleges that the general guidelines provided in the specification for generating siRNAs "do not address the particulars of the siRNA design and selection process required for obtaining siRNAs against any mutant allele such that one of ordinary skill in the art reading the specification at the time of filing could envision any siRNA targeted to an vast number of mutant alleles responsible for gain-of-function diseases." Contrary to the Examiner's allegation, the specification provides extensive guidance for designing and selecting the sequences of siRNAs which can be used for allele-specific cleavage in the present invention.

For example, Applicants provide a plethora of explicit examples of diseases caused by dominant, gain-of-function gene mutations, including Alzheimer's disease, Huntington's disease, Parkinson's disease and ALS (***see, e.g., page 1, second paragraph; page 18, line 12 through page 19, line 21; and references 1 and 41-56 as listed at pages 30-35 of the specification, all of which are incorporated by reference***). One of ordinary skill in the art

would recognize that the sequences of the mutant alleles responsible for these diseases were common knowledge in the art and easily obtainable at the time of filing the instant application.

Further, the specification provides detailed guidance for the process of designing siRNA molecules to target a particular mutant allele (see, for example, pages 8-16 and, in particular, pages 10-11):

> 1. Beginning with an AUG start codon, search for AA dinucleotide sequences; each AA and the 3' adjacent 16 or more nucleotides are potential siRNA targets. ***The siRNA should be specific for a target region that differs by at least one base pair between the wild type and mutant allele, e.g., a target region comprising the gain-of-function mutation. In cases where the gain-of-function mutation is associated with one or more other mutations in the same gene, the siRNA can be targeted to any of the mutations.*** In some cases, the siRNA is targeted to an allelic region that does not comprise a known mutation but does comprise an allelic variation of the wild-type (reference) sequence. The first strand should be complementary to this sequence, and the other strand is identical or substantially identical to the first strand. ***In one embodiment, the nucleic acid molecules are selected from a region of the target allele sequence beginning at least 50 to 100 nt downstream of the start codon, e.g., of the sequence of SOD1***. Further, siRNAs with lower G/C content (35-55%) may be more active than those with G/C content higher than 55%. Thus in one embodiment, the invention includes nucleic acid molecules having 35-55% G/C content. In addition, the strands of the siRNA can be paired in such a way as to have a 3' overhang of 1 to 4, *e.g.*, 2, nucleotides. Thus in another embodiment, the nucleic acid molecules can have a 3' overhang of 2 nucleotides, such as TT. The overhanging nucleotides can be either RNA or DNA.
>
> 2. Using any method known in the art, compare the potential targets to the appropriate genome database (human, mouse, rat, etc.) and eliminate from consideration any target sequences with significant homology to other coding sequences. One such method for such sequence homology searches is known as Basic Local Alignment Search Tool (BLAST), which is available at the National Institutes of Health (NIH)/National Library of Medicine's (NLM's) National Center for Biotechnology Information (NCBI) website.
>
> 3. Select one or more sequences that meet your criteria for evaluation. Further general information about the design and use of siRNA may be found in "The siRNA User Guide," available at the Max Planck Institute for Biophysical Chemistry website.

The specification further provides detailed guidance for selecting the appropriate modified bases and the positions at which modified bases are placed in the siRNAs of the invention. For example, the specification teaches that

> [w]here the mutation results in the ***replacement of a base in the target mRNA with an adenine***, siRNAs modified with ***U(5Br) or U(5I) in the antisense strand are generally used***. Where the mutation results in ***the replacement of a base target RNA with a uracil*** (***thymine*** in the DNA), siRNAs modified with ***DAP in the antisense strand are generally used***. (See pages 7-8, bridging paragraph).

Finally, Applicants' specification provides ample guidance for how to test and select siRNA molecules based on their ability to inhibit target mRNA expression, *e.g.*, in Examples 1-3 at pages 28-30 of the specification. Moreover, such techniques were routine in the art at the time of filing of the present application.

In view of the foregoing, it is evident that one of ordinary skill in the art would have recognized that, based on the teachings in the present specification, Applicants were in possession of the claimed invention at the time of filing.

Moreover, Applicants submit that, at the time the present application was filed, the existing knowledge in the siRNA field and the maturity of the RNAi technology was great. Indeed, it was common knowledge in the siRNA field how to go about selecting one or more specific target sequences in a target mRNA (*e.g.*, as described in detail by Applicants, as set forth above), how to generate one or more siRNA molecules targeted to that particular mRNA sequence and, moreover, how to test a panel of siRNA molecules for the ability to inhibit expression of the target mRNA. Such techniques were routine to one of ordinary skill in the art at the time the present application was filed. Further, the level of skill in the art with respect to incorporating modified nucleotides into RNA was similarly high at the time of filing the present application.

Accordingly, for at least the foregoing reasons, it would have been clear to one of ordinary skill in the art, based on the present specification and the high level of skill in the relevant art, that Applicants' had full possession of the claimed invention at the time of filing. Applicants therefore respectfully request the Examiner to reconsider and withdraw this rejection under 35 U.S.C. §112, first paragraph.

Rejection of Claims 2-8 and 10-17 under 35 USC § 103(a)

The Examiner has rejected claims 2-8 and 10-17 under 35 U.S.C. §103(a) as being obvious over Ecker *et al.* (US Patent No. 5,965,722) in view of Hojo *et al.* (Eur. Respir. J. 1998), Hammond *et al.* (Nature Reviews Genetics 2001), Bass *et al.* (Nature 2001) and Tuschl *et al.* (WO 02/44321). The Examiner relies on Ecker *et al.* for teaching that "antisense compounds comprising modified nucleotide bases increase the affinity for base mismatches in mutated genes and further enhance the compounds selectivity for such mutated genes." The Examiner further relies on Ecker *et al.* for teaching that "a single nucleotide mutation is responsible for mutated Ras protein expression," that "incorporation of a 2'amino adenine at the position that is complementary to the uracil of the mutated codon serves to stabilize the hybridization of the antisense oligonucleotide to the mutated gene," and that "incorporation of a 2,6-diamino adenosine complementary to the uracil of the mutated codon was also found to be effective in increasing the hybridization of the antisense compound to the mutated gene." The Examiner acknowledges that Ecker *et al.* "***do not teach siRNA targeted to a mutated gene*** and do not teach the point mutation is an adenine or thymine" (emphasis added).

The Examiner relies on Hojo *et al.* for teaching that lung cancer is associated with overexpression of p53 and that "overexpression of p53 is due to point mutations of the p53 gene wherein the mutations are commonly an adenine or a thymidine."

The Examiner relies on Hammond *et al.* for teaching that "antisense methods... have suffered from '...questionable specificity and incomplete efficacy,'" that "...dsRNAs have been shown to inhibit gene expression in a sequence-specific manner" and that "RNAi is a potent method, requiring only a few molecules of dsRNA per cell to silence expression." Similarly, the Examiner relies on Bass *et al.* for teaching that siRNA has "... repeatedly proven itself to be more robust than antisense techniques: it works more often, and typically decreases expression of a gene to lower levels, or eliminates it entirely."

The Examiner relies on Tuschl *et al.* for teaching that "siRNA may contain at least one modified analogue, such as a modified base wherein the modified base comprises 5-bromouracil or 5-iodouracil, and the modification may be located at positions that do not interfere with RNAi mediating activity."

The Examiner alleges that "Ecker *et al.* provide evidence that one of skill in the art would have had a reasonable expectation of inhibiting a mutant target gene," and "given that

Tuschl *et al.* teach how to make and use any siRNA targeted to any gene, [and that] Hammond *et al.* and Bass *et al.* teach siRNA are preferred over antisense compounds, one would have had a reasonable expectation of success at making a siRNA targeted to a mutated gene." The Examiner concludes that "the invention would have been prima facie obvious to one of skill in the art."

Applicants respectfully traverse the rejection based on the following arguments.

Claim 2 (and the claims that depend therefrom) is directed to an ***siRNA*** capable of single nucleotide discrimination between a first and second allele, the first allele having 1, 2, 3 or more point mutations relative to the second allele, wherein the ***siRNA comprises a sense strand and an antisense strand***, wherein the antisense strand comprises a ***modified base positioned opposite at least one point mutation in the first allele***, and wherein the modified base is capable of enhancing binding interactions between the siRNA and mRNA encoded by the first allele when compared with binding interactions between the siRNA and mRNA encoded by the second allele. Claim 3 (and the claims that depend therefrom) is drawn to an ***siRNA comprising a sense strand and an antisense strand***, wherein the sense strand comprises a sequence homologous to a region of a mutant allele encoding a gain-of-function mutant protein, said region comprising one or more point mutations, and wherein the ***antisense strand comprises a sequence comprising one or more modified bases positioned opposite the point mutations***, such that the ***siRNA directs allele-specific cleavage*** of a mRNA encoded by the mutant allele.

The teachings of Ecker *et al.* are directed to ***antisense DNA oligonucleotides*** for specific inhibition of expression of an activated, or mutant, form of the ras gene. In particular, Ecker *et al.* teach that antisense phosphorothioate DNA oligononucleotides in which a modified nucleotide (*i.e.*, 2-(amino)adenine or 2,6-(diamino)adenine) is positioned complementary to the uracil of the mutated codon 12 of activated ras stabilize hybridization of the modified antisense oligonucleotide to the activated ras gene and increase specificity for the mutant target. Ecker *et al.* fail to teach or suggest ***any RNA oligonucleotide***, let alone a double stranded siRNA molecule targeting a mutant allele. Indeed, nothing in the teachings of Ecker *et al.* suggests a need in the art for an alternate molecule to an antisense molecule for the specific inhibition of expression of a mutant gene, let alone an siRNA. Thus, based on the teachings of Ecker *et al.*, one would not be motivated to seek alternate molecules to antisense for specific mutant gene silencing.

Moreover, Ecker *et al.* ***teach away*** from the claimed invention. In particular, Ecker *et al.* teach that RNase H is an endonuclease that cleaves the RNA strand of RNA:DNA duplexes and that activation of this enzyme by an antisense oligonucleotide results in cleavage of the RNA target (column 4, lines 37-41). Ecker *et al.* teach, therefore, that preferred antisense oligonucleotides are ***DNA oligonucleotides,*** *e.g.*, having phosphodiester or phosphorothioate inernucleoside linkages, since they activate the cleavage of target RNA by RNase H and "greatly enhance the ability of antisense oligonucleotides to inhibit target RNA expression" (column 4, lines 28-42). Accordingly, one would not have been motivated, based on the teachings of Ecker *et al.*, to substitute the antisense DNA oligonucleotides of Ecker *et al.* with an RNA oligonucleotide, since Ecker *et al.* teach that an RNA oligonucleotide would fail to activate RNase H and, thus, fail to inhibit expression of target RNA efficiently.

The teachings of Hojo *et al.* fail to make up for the deficiencies of Ecker *et al.* Hojo *et al.* teach that pulmonary fibrosis is associated with overexpression of p53, and that such p53 overexpression is often associated wtih guanine(G):cytosine(C) to adenine(A):thymine(T) and A:T to G:C transitions. Hojo *et al.* fail to teach or suggest anything regarding siRNAs, let alone siRNAs having a modified nucleotide in the antisense strand positioned opposite a point mutation in a mutant allele, as presently claimed.

The teachings of Hammond *et al.* and Bass *et al.* also fail to make up for the deficiencies of Ecker *et al.* Both the Hammond and Bass references generally teach that RNAi is an effective means of silencing genes, and that RNAi is more "robust" than antisense technologies. Both the Hammond and Bass references fail to teach or suggest siRNAs comprising <u>any</u> modified nucleotides, let alone modified nucleotides positioned opposite point mutations in mutant alleles, nor do these references teach that such modified siRNAs may be used to achieve single nucleotide discrimination between a wild type and mutant allele.

Finally, Tuschl *et al.* fails to make up for the deficiencies of Ecker *et al.* Tuschl *et al.* is generally directed to siRNA molecules useful for mediating RNA interference. Tuschl *et al.* disclose that the ***stability of siRNAs against degradation*** may be enhanced by the "substitution of pyrimidine nucleotides by modified analogues" (see page 5, first paragraph). Tuschl *et al.* disclose that these modified pyrimidine analogues may be nucleobase-modified ribonucleotides, including "uridines or cytidines modified at the 5-position, *e.g.*, ... 5-bromo uridine" (see page 5, third paragraph) and that these modifications "may be located at positions that do not interfere with RNAi mediating activity." As set forth in Applicant's previous Response filed February

15, 2007, Tuschl *et al.* fail to teach both the ***structure*** and the ***function imparted by that structure*** of the presently claimed siRNAs. In particular, Tuschl *et al.* fail to teach or suggest the specific positioning of a modified base in the antisense strand of an siRNA opposite a point mutation in the target mRNA of a mutant allele. Tuschl *et al.* also fail to teach or suggest that a modified base at any position in an siRNA, let alone at the position required by the pending claims, is useful for enhancing binding interactions between the siRNA and an mRNA encoded by a first allele as compared to a second allele, when the first allele has one or more point mutations relative to the second allele. Indeed, Tuschl *et al.* fail to teach or suggest an siRNA comprising a modified base for any purpose other than to ***increase stability against degradation***.

In view of the foregoing, one of ordinary skill in the art would not have been motivated to combine the teachings of the cited art to arrive at the present invention, as currently claimed, in view of the art at the time of filing. A case of *prima facie* obviousness can only be established by combining or modifying the teachings of the prior art to produce the claimed invention where there is some teaching, suggestion, or motivation to do so found either explicitly or implicitly in the references themselves or in the knowledge generally available to one of ordinary skill in the art. The Examiner, however, has not established a *prima facie* case of obviousness as there is nothing in the teachings of Ecker *et al.,* Hojo *et al,* Hammond *et al.,* Bass *et al.* or Tuschl *et al.* that would motivate one of ordinary skill in the art to combine the cited prior art references, let alone to combine them in such a way as to arrive at the claimed invention.

Moreover, even if the motivation to extend the teachings of Ecker *et al.* to an RNAi-based approach were to exist, (which it does not), the skilled artisan would have had no reasonable expectation of success in using the Ecker methodology. The skilled artisan would have readily understood, at the time the instant application was filed, that the results obtained for targeting an ***antisense molecule*** containing modified nucleotides to a mutated gene (as taught by Ecker *et al.*) cannot be extrapolated to the targeting of an ***siRNA molecule*** to a mutant allele, where the siRNA comprises an antisense strand having a modified nucleotide positioned opposite the point mutation of the mutant allele, with any reasonable expectation of success because the molecules operate through very different cellular mechanisms. In particular, the state of the art at the time of filing recognized that an antisense oligonucleotide inhibits transcription and/or translation of target genes by base-pairing with the target sequence and blocking translocation of the transcription/translation machinery. In contrast, RNAi was recognized to involve the assembly of the RNA molecule with protein components to form a

nuclease complex, RNAi-Inducing Silencing Complex (RISC), that RISC utilizes an active mechanism to search for the homologous mRNA target and ultimately mediates degradation of the mRNA target. Given the distinct mechanism of RNAi as compared to that of antisense technology, the skilled artisan would not, based on the current state of the art and the teachings of the cited references, have had any reasonable expectation of success in making and using an siRNA as claimed. One skilled in the art would recognize that an antisense molecule and an siRNA cannot be used interchangeably because they operate through very different mechanisms, and thus the successful substitution of an siRNA in the teachings of Ecker *et al.* is not predictable.

In particular, as noted by the Examiner, and as evidenced by the Hammond *et al.* and Bass *et al.* references, RNAi is a ***remarkably efficient means of effecting gene silencing as compared to antisense technology***. Indeed, the state of the art at the time of filing of the instant application recognized that siRNAs containing single-base mismatches with the target RNA could effectively mediate RNAi silencing of the target gene. Accordingly, one of skill in the art would not expect that single-nucleotide discrimination between a wild type and mutant allele obtained with an ***antisense oligonucleotide***, where the discrimination results solely from the presence of a single modified nucleotide positioned opposite a point mutation of the mutant gene, could be extrapolated to an ***siRNA*** having a single modified nucleotide similarly positioned opposite the point mutation of a mutant gene. There is nothing in Ecker *et al.* that teaches or even remotely suggests that a wild type gene would be ***resistant to RNAi.*** Ecker *et al.* merely teaches that the wild type ras gene, as compared to mutant ras, is resistant to the relatively inefficient antisense silencing technology of the reference. Notably, the siRNA methodology of the instant invention significantly silences a target mutant gene as compared to the corresponding wild type gene.

In summary, the Examiner has failed to point to any teaching in the Ecker *et al.*, Hojo *et al.*, Hammond *et al.*, Bass *et al.* and Tuschl *et al.* references that would compel one of ordinary skill in the art to make the claimed invention. The prior art must suggest "to those of ordinary skill in the art that they ***should*** make the claimed composition or device, or carry out the claimed process" and ***[b]oth the suggestion*** and the ***reasonable expectation of success*** must be founded ***in the prior art, not in the applicant's disclosure*** (emphasis added)." *In re Dow Chemical Co.* 837 F.2d 469, 473, 5 U.S.P.Q.2d 1529, 1531 (Fed. Cir. 1988). In view of the foregoing, Applicants request that the rejection of claim 14 under § 103(a) be reconsidered and withdrawn.

Rejection of Claims 2-5, 7 and 9-17 under 35 USC § 103(a)

The Examiner has rejected claims 2-5, 7 and 9-17 under 35 U.S.C. §103(a) as being obvious over Ecker *et al.* (US Patent No. 5,965,722), Hammond *et al.* (Nature Reviews Genetics 2001), Bass *et al.* (Nature 2001), Tuschl *et al.* (WO 02/44321) and Xu *et al.* (US 2004/0192629).

The Examiner relies on Ecker *et al.* for the reasons set forth above. The Examiner acknowledges that Ecker *et al.* "do not teach siRNA targeted to a gene correlated with a disease selected fro[m] ALS, Huntington's disease, Alzheimer'[s] disease or Parkinson's Disease."

The Examiner relies on Hammond *et al,* Bass *et al.*, and Tuschl *et al.* for the reasons set forth above. Finally, the Examiner relies on Xu *et al.* for teaching that "allele-specific RNA interference of mutated genes comprises administering a siRNA targeted to the mutant gene," that "certain types of human disease, such as ALS, Hungtington's disease, Alzheimer'[s] disease or Parkinson's Disease are caused by dominant gain-of-function mutations, and because the wild-type gene often performs important functions compared to the toxic effects of the mutant gene, it would be advantageous to selectively inhibit the mutated gene.""

The Examiner alleges that "Ecker *et al.* provide evidence that one of skill in the art would have had a reasonable expectation of inhibiting a mutant target gene" and "given that Tuschl *et al.* teach how to make and use any siRNA targeted to any gene, [and] Hammond *et al.* and Bass *et al.* teach siRNA are preferred over antisense compounds, one would have had a reasonable expectation of success at making a siRNA targeted to a mutated gene." The Examiner further alleges that "[o]ne of skill in the art would have had a reasonable expectation of success at targeting a gene responsible for disorders such as ALS given that Xu *et al.* teach a

specific embodiment of selective targeting a mutant gene of ALS while not targeting the wild-type gene." The Examiner concludes that "the invention would have been prima facie obvious to one of skill in the art."

As set forth above, the teachings of Ecker *et al.* are directed to ***antisense DNA oligonucleotides*** comprising a modified nucleotide positioned opposite uracil of the mutated codon 12 of activated ras for the specific silencing of the mutant ras gene. Ecker *et al.* fail to teach or suggest ***any RNA oligonucleotide***, let alone a double stranded siRNA molecule targeting a mutant allele. Nothing in the teachings of Ecker *et al.* suggests a need in the art for an alternate molecule to an antisense molecule for the specific inhibition of expression of a mutant gene, let alone an siRNA. Thus, based on the teachings of Ecker *et al.*, one would not be motivated to seek alternate molecules to antisense for specific mutant gene silencing. Moreover, Ecker *et al.* ***teach away*** from the claimed invention. Ecker *et al.* teach, therefore, that preferred antisense oligonucleotides are ***DNA oligonucleotides*** (*e.g.*, having phosphodiester or phosphorothioate inernucleoside linkages), since they activate the cleavage of target RNA by RNase H and "greatly enhance the ability of antisense oligonucleotides to inhibit target RNA expression." Accordingly, one would not have been motivated, based on the teachings of Ecker *et al.*, to substitute the antisense DNA oligonucleotides of Ecker *et al.* with an RNA oligonucleotide, since Ecker *et al.* teach that an RNA oligonucleotide would fail to activate RNase H and, thus, fail to inhibit expression of target RNA efficiently.

The teachings of Hammond *et al.*, Bass *et al.* and Tuschl *et al.* fail to make up for the deficiencies of Ecker *et al.* As set forth above, both the Hammond and Bass references fail to teach or suggest siRNAs comprising <u>any</u> modified nucleotides, let alone modified nucleotides positioned opposite point mutations in mutant alleles, nor do these references teach that such modified siRNAs may be used to achieve single nucleotide discrimination between a wild type and mutant allele. Also as discussed above, Tuschl *et al.* fail to teach or suggest an siRNA comprising a modified base for any purpose other than to ***increase stability against degradation***. In particular, Tuschl *et al.* fail to teach or suggest the specific ***positioning*** of a modified base in the antisense strand of an siRNA opposite a point mutation in the target mRNA of a mutant allele. Tuschl *et al.* also fail to teach or suggest that a modified base at ***any*** position in an siRNA is useful for enhancing binding interactions between the siRNA and an mRNA encoded by a first allele as compared to a second allele.

Finally, Xu *et al.* fail to make up for the deficiencies of Ecker *et al.* As discussed in Applicant's previous Response filed February 15, 2007, Xu *et al.* (*i.e.*, priority document 60/423,507, filed November 4, 2002, the only Xu *et al.* priority document which has a filing date preceding the priority date of the instant claims) teach preferential gene silencing of the mutant sod1 gene, relative to wild type sod1, with an siRNA, wherein the antisense strand of the siRNA comprises ***standard nucleotides*** and has a sequence perfectly complementary to the target mRNA sequence. The Xu *et al.* priority document 60/423,407 fails to teach an siRNA comprising a modified base positioned opposite a point mutation in a mutant allele, as required by the pending claims.

In view of the foregoing, one of ordinary skill in the art would not have been motivated to combine the teachings of the cited art to arrive at the present invention, as currently claimed, in view of the art at the time of filing. The Examiner has not established a *prima facie* case of obviousness as there is nothing in the teachings of Ecker *et al.*, Xu *et al,* Hammond *et al.*, Bass *et al.* or Tuschl *et al.* that would motivate one of ordinary skill in the art to combine the cited prior art references, let alone to combine them in such a way as to arrive at the claimed invention.

Moreover, as discussed above, even if the motivation to extend the teachings of Ecker *et al.* to an RNAi-based approach were to exist, (which it does not), the skilled artisan would have had no reasonable expectation of success in using the Ecker methodology. The skilled artisan would have readily understood, at the time the instant application was filed, that the results obtained for targeting an ***antisense molecule*** containing modified nucleotides to a mutated gene (as taught by Ecker *et al.*) cannot be extrapolated to the targeting of an ***siRNA molecule*** to a mutant allele, where the siRNA comprises an antisense strand having a modified nucleotide positioned opposite the point mutation of the mutant allele, with any reasonable expectation of success because the molecules operate through very different cellular mechanisms. In particular, the state of the art at the time of filing recognized that an antisense oligonucleotide inhibits transcription and/or translation of target genes by base-pairing with the target sequence and blocking translocation of the transcription/translation machinery. In contrast, RNAi was recognized to involve the assembly of the RNA molecule with protein components to form a nuclease complex, RNAi-Inducing Silencing Complex (RISC), that RISC utilizes an active mechanism to search for the homologous mRNA target and ultimately mediates degradation of

the mRNA target. Given the distinct mechanism of RNAi as compared to that of antisense technology, the skilled artisan would not, based on the current state of the art and the teachings of the cited references, have had any reasonable expectation of success in making and using an siRNA as claimed. One skilled in the art would recognize that an antisense molecule and an siRNA cannot be used interchangeably because they operate through very different mechanisms, and thus the successful substitution of an siRNA in the teachings of Ecker *et al.* is not predictable.

In particular, as noted by the Examiner, and as evidenced by the Hammond *et al.* and Bass *et al.* references, RNAi is a ***remarkably efficient means of effecting gene silencing as compared to antisense technology***. Indeed, the state of the art at the time of filing of the instant application recognized that siRNAs containing single-base mismatches with the target RNA could effectively mediate RNAi silencing of the target gene. Accordingly, one of skill in the art would not expect that single-nucleotide discrimination between a wild type and mutant allele obtained with an ***antisense oligonucleotide***, where the discrimination results solely from the presence of a single modified nucleotide positioned opposite a point mutation of the mutant gene, could be extrapolated to an ***siRNA*** having a single modified nucleotide similarly positioned opposite the point mutation of a mutant gene. There is nothing in Ecker *et al.* that teaches or even remotely suggests that a wild type gene would be ***resistant to RNAi.*** Ecker *et al.* merely teaches that the wild type ras gene, as compared to mutant ras, is resistant to the relatively inefficient antisense silencing technology of the reference. Notably, the siRNA methodology of the instant invention significantly silences a target mutant gene as compared to the corresponding wild type gene.

In summary, the Examiner has failed to point to any teaching in the Ecker *et al.*, Xu *et al.*, Hammond *et al.*, Bass *et al.* and Tuschl *et al.* references that would compel one of ordinary skill in the art to make the claimed invention. The prior art must suggest "to those of ordinary skill in the art that they ***should*** make the claimed composition or device, or carry out the claimed process" and ***[b]oth the suggestion*** and the ***reasonable expectation of success*** must be founded ***in the prior art, not in the applicant's disclosure*** (emphasis added)." *In re Dow Chemical Co.* 837 F.2d 469, 473, 5 U.S.P.Q.2d 1529, 1531 (Fed. Cir. 1988). In view of the foregoing, Applicants request that the rejection of claim 14 under § 103(a) be reconsidered and withdrawn.

In view of the above amendments and remarks, it is believed that this application is in condition for allowance. If a telephone conversation with Applicants' Attorney would expedite prosecution of the above-identified application, the Examiner is urged to call the undersigned at (617) 227-7400.

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Respectfully submitted,

By _____

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